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The Effects of Extremely Low-Frequency Magnetic Fields on Reproductive Function in Rodents

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Abstract

Extremely low-frequency electromagnetic fields (ELF-EMF) are defined as those having frequencies up to 300 Hz, representing a non-ionising radiation having photon energy too weak to interact with biomolecular systems. Exposure to low-frequency electric field and magnetic field (MF) generally results in negligible energy absorption in the body. However, it is well established that ELF-MF induces biologic effects in various cellular functions. ELF-MF acting as a co-inducer can potentiate weak mutagenic signalling. The concern about possible adverse effects on human health of long-term exposure to ELF-MFs, especially at frequencies of 50 or 60 Hz generated from power lines and electric devices, has been increasing. Conversely, long-term effects of chronic exposure have been excluded from the scope of the guidelines of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) because of insufficient consistent scientific evidence to fix the thresholds for such possible biological effects. The results regarding the adverse effects of ELF-MF on human or animal reproductive functions are contradictory or inconclusive. Overall conclusion of epidemiologic studies on ambient residential MF exposure consistently failed to establish a link between human adverse reproductive outcomes and chronic maternal or paternal exposure to low-frequency MFs. In animal studies, there is no compelling evidence for a causal relationship between disturbed prenatal development and ELF-MF exposure. Testicular spermatogenesis progresses through a complexly regulated cellular process involving mitosis and meiosis; this process seems to be vulnerable to external stressors, such as heat, MF exposure or chemical and physical agents. Exposure to ELF-MF did significant risk impaired implantation or the foetal development in animal studies. However, there is some consistency in the increase of minor skeletal alterations in animal experiments. The evidence derived from recent studies in male mice demonstrates that ELF-MF exposure is involved with an increase in the frequency of apoptosis in spermatogenic cells. Those results suggest that exposure to MF is related to possible cytogenetic effects on testicular germ cells and therefore may negatively affect reproduction. This chapter intends to present an overview on the effects of ELF-EMF exposure on the reproductive function and a plausible mechanism in rodent species.

Keywords: ELF-MF, 60 Hz, Reproduction, Germ cell apoptosis, Disruptors

1. Introduction

Life including human on earth has evolved in and adapted to the environment of various natural electromagnetic fields (EMFs) with relatively weak energy. In the last century, man-made EMFs with various spectrums were introduced into the natural environment. Long-term effects of man-made EMF on human health are not established. Human-made EMF is classified into three categories: low-frequency (LF) fields (1 Hz–100 kHz), high-frequency fields in the band of radiofrequency (100 kHz–3 GHz) and microwaves (above 3 GHz). Extremely low-frequency electromagnetic fields (ELF-EMF) are defined as those having frequencies up to 300 Hz. Ambient ELF-magnetic field (MF) is generally generated by the electric power transmission as alternating current at 50 or 60 Hz. The exposure to ELF-MF is increasing as a consequence of the wide use of electricity and electrical appliances at home or in the workplace. Therefore, it is a growing concern whether human-made EMF induces biological effects that might be harmful to human health.

For the induction of biological effect of ELF-MF, the MF is more deleterious than the electric field (EF) because MF induces an electric current in the body, while EF does not [1]. The direct biological effects of an electromagnetic field are divided into thermal effects by electromagnetic field energy absorption, stimulation function by induced electrical currents and non-thermal action by long-term exposure [2, 3]. The mechanisms of biological effects differ according to the varying frequency of EMF. Thermal effects mainly occur over 100 kHz radiofrequency. Despite statistical association between ambient residential MF exposure and childhood cancer in epidemiologic investigations [4–6] and scientific results of EMF relating to genotoxic effects [7–9], there is no plausible mechanism of cancer and no evidence for cancer in adults.

MF alone has generally not been related to genetic damage [10, 11]. However, MF exposure might enhance the effects of known DNA-damaging agents [12]. The International Agency for Research on Cancer (IARC) has classified it with 2B possibly carcinogenic to humans—based on the epidemiologic results on childhood leukaemia [10].

Available evidences are insufficient to confirm the effect of 50/60-Hz EMF generated by power lines and electric devices on human health. The magnitude and distribution of MF currents depend on the frequency, the size of the object and proximity between the objects and a conducting device [1]. In general, the conclusions of epidemiologic studies have not consistently demonstrated the association between human adverse reproductive outcome and maternal or paternal exposure to low-frequency magnetic fields. A meta-analysis failed to demonstrate an increased risk of spontaneous abortion or malformation in studies comparing pregnant woman using a video display terminal with those not using it [13]. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines are based on short-term, immediate health effects such as peripheral nerve, muscle, burn and elevated tissue temperature [1]. Long-term effects of chronic exposure have been excluded from the scope of the ICNIRP guidelines because of insufficiently consistent scientific evidence to fix the thresholds for such putative biological effects.

In animal studies, exposure to ELF-MF does not significantly affect implantation and the development of a foetus [14–20] but may induce foetal death, congenital abnormalities, minor

skeletal anomalies and a decrease in the number of foetus impregnated by exposed males [21–23]. There is increasing evidences from animal studies of adverse effects of exposure to ELF-MF on the male reproductive system, such as a decrease in sperm number and testis volume [24], an increase in the frequency of apoptosis of spermatogenic cells [25–27], a significant decrease in the diameter of seminiferous tubules [28] and an alteration of the pituitary–gonadal axis [29–31]. Conversely, other studies report that ELF-MF exposure has no adverse effects on the reproductive function in animal [32–34], although in those studies the daily exposure was relatively short.

Superficially located testes could be more affected by MF than the internal organs. The testis is the most sensitive tissue for thermal thresholds compared with other tissues, such as the spinal cord, intestines or skin [35]. Testicular spermatogenesis is a complex process comprising the transformation from spermatogonia, primary spermatocyte, secondary spermatocyte, round spermatid and elongated spermatid to sperm through a series of events involving mitosis, meiosis and cellular differentiation [36]. That makes the testis one of the most vulnerable organs in the whole body to external stressors, such as heat, MF or chemical agents. The thermal thresholds for tissue damage vary with the animal species and tissue; for example, tissue damage in mice is lower compared to humans and pigs [35]. The mechanisms involved in the reported adverse effects on reproductive function remain unclear. To date, the contradictory results of outcome regarding the biological effect of ELF-MF exposure seem to be related to the variability of exposure system, exposure conditions including intensity of MF and exposure duration and experimental animal including species and age. Thus, reproducibility of the study on EMF is almost unsuccessful in independent laboratories. Therefore, it is not easy to find the causal relationship between ELF-EMF exposure and experimental results. This chapter describes the overall effect of ELF-MF exposure on the reproductive function and biologic effect in mice or rats on the basis of reported scientific literatures.

2. ELF-MF exposure and epidemiologic study in human

Certain epidemiologic studies demonstrate that exposure to ELF-MF may lead to an increased risk of certain types of adult and childhood cancer, including leukaemia, cancer of the central nervous system and lymphoma [4–6]. However, others failed to find such an association [37–39]. The results of epidemiologic studies on the effects of ELF-EMF on reproductive function have been contradictory since 1986, when it was reported that electric blankets and heated water usage may increase the abortion rate and underweight delivery [4]. The possible effects of heat cannot be linked to those of EMF. The epidemiology study investigating the reproductive effect of residential exposure to ELF-MF has not found a relationship between MF and reproductive outcomes, such as foetal loss, pregnancy loss and miscarriage [40–43]. Two prospective studies show that no association exists between low birth weight or the rate of spontaneous abortion and the use of electric bed heaters [42, 43].

The limitation in most of the studies was that measurement of ELF field density was not included. Field strength of residential ELF-MF has been reported to vary between 0.05 and

0.11 μT in the USA and between 0.025 and 0.07 μT in Europe [44]. The results are inconclusive due to potential confounders and the low number of cases [40]. It was proposed that good practice for human studies should include a double-blind design, appropriate criteria for inclusion and exclusion of volunteer [45]. According to the ICNIRP guideline for limiting exposure to time-varying EMF (1 Hz to 100 kHz), the overall conclusion of epidemiologic studies shows no consistent association between human adverse reproductive outcomes and maternal or paternal exposure to low-frequency fields [1].

3. Effects of ELF-MF exposure on reproductive function

3.1. Effects on prenatal development: Teratologic studies

Various reports showed that male and female mice exposure to ELF-MF has no significant risk on fertility and on the reproductive function in mice. In mammals, prenatal exposure to ELF does not increase miscarriage and gross external, visceral or skeletal malformations using fields up to 20 mT strength [14, 16–18, 21]. No significant differences on testis volume and sperm parameters were observed in male offspring of pregnant rats exposed to a field density of 0.83 or 500 μT until 21 days of lactation [18]. On the contrary, others report that a significant decrease in the number of implantations and living foetus per litter was observed in male and female rats exposed to 50-Hz MF of 25 μT for 90 days before mating [23].

The increase in the development of minor skeletal anomalies has been consistently reported in mouse or rat experiments [15, 16, 21, 22] (Table 1). The lowest field density to induce skeletal alteration was 13 μT [22]. Since the skeletal alteration is a common finding for prenatal exposure and may result from statistical fluctuation, it may be considered biologically insignificant [46]. Interestingly, in a toxicity study of harmonics MF, exposure to 180-Hz MF in combination with 60-Hz MFs had no significant effects on litter size, litter weight or live birth rates but induced an increase in the incidence of rib variants. Nevertheless, the incidence was not significantly different from that in controls exposed to 60 Hz alone [16]. In rats, prenatally exposed to 60 Hz of field strength 1 mT, it was described the existence of certain alterations in testicular histology, such as decreased in height of seminiferous epithelium, an increased in size of Leydig cell and of connective tissue in the testis [47], suggesting that exposure to ELM-MF may be a risk factor for reproductive function. Still, prenatal exposure to ELF-MF in Wistar rat was not a biological significant risk factor for foetal development. Table 1 lists the reports regarding effects of ELM-MF on foetal development.

3.2. Multigenerational studies

Few multigenerational studies have been reported [19, 48]. In a Sprague-Dawley rats study encompassing three generations, continuous exposure to 60-Hz MF for 18.5 h per day at field strengths of 0, 2, 200 or 1000 μT or to an intermittent MF (1 h on/1 h off) at 1000 μT was performed. No significant exposure-related adverse effects were found in all three generations with respect to the reproductive function, namely in litter/breeding pair, percentage of fertile

MF (Hz)	Animal	Age	Intensity	Exposure	Duration	Skeleton anomalies	Effects on reproduction & anomaly	Result	Ref.
50	CD1 mice	Pregnant	20 mT	0–17 days	24 h	Non	Abortion rate congenital malformation	NS	14 (Kowalczuk et al, 1994)
50	Wistar rat	Pregnant	30 mT	1–29 days	24 h	Skeletal ossification ↑	No congenital malformation	S	15 (Mevisen et al, 1994)
60 or +180	SD rat	Mated female	0.2 mT	6–19 days	18.5 h/day	Rib variants	Litter size, litter weight or foetal development (↔)	NS	16 (Ryan et al, 2000)
50	Swiss mice M, F	60 days, before mate	25 µT	90 days	24 h	Non	Implantation site, viable foetus, number of resorption, testis weight (↔) and ovary weight ↑	NS	17 (Elbetieha et al, 2002)
60	SD rat	Pregnant female	0.83, 3, 500 µT	Gestation 6 days to lactation 21 days	21 h/day	Non	Litter size, anogenital distance, testis weight, sperm parameter (↔)	NS	18 (Chung et al, 2003)
50	Wistar rat	Mated female	35 µT	0–20 days	24 h	Skeletal anomaly ↑	Pregnancy rates, incidences of resorptions, late foetal deaths (↔)	S	21 (Huuskonen et al, 1993)
50	CBA/Ca mice	Mated female	13 µT	0–18 days	24 h	Skeletal anomaly ↑	Malformation/resorption (↔)	S	22 (Huuskonen et al, 1998)
50	B6C3F1 mice	Pregnant, F1	50 µT	7 days, F1 15.5 ms	12 h/day		Seminiferous tubule size ↓, in female, leukaemia ↑	S	28 (Qi et al, 2015)

NS, not significant; S, significant.

Table 1. Effects of ELF-MF on prenatal development.

pair, latency to parturition or litter size [48]. In contrast, another study using continuous exposure to 60-Hz MF at a field strength of 0.5 and 1.5 mT in three generations showed a consistent reduction of weight of the ovary and testis in F2 mice, although no significant effects were found on implantation. However, no significant difference of testis weight was observed in F3 male mice [19]. Interestingly, it was observed in F1 and F2, but not in F3 mice, an increased frequency of a certain type of tumours including lymphoma, adenocarcinoma or benign tumour compared with those in the control group. The results suggest that EMF exposure

induces possible cytogenetic effects on living cells including gonadal cells and the biological adaptation for chronic exposure to ELF-MF may take place.

4. Effect on sperm count and testis weight

The testis volume reflects the activity of spermatogenesis in seminiferous tubules. The lumen diameter of the seminiferous tubule may be regulated by elongated spermatids in rats [49]. The reduction in the testicular volume generally indicates impairment of spermatogenesis.

It is well known that an ELF-MF with weak energy has a significant cytogenetic effect on spermatogenic cells in the testis. It has reported a significant decrease in the counts for mature spermatid or epididymal sperm and the alteration in sperm parameters in mice or rats chronically exposed to ELF-MF [24–27, 31, 50, 51] (Table 2). Recently, it was shown that chronic exposure to ELF-MF is related to a significant risk for chronic myeloid leukaemia in female and a decrease in the size of seminiferous tubules in male mice [28]. Testis weight was significantly lower than the control in accordance to a decreased sperm count [24, 29]. On the contrary, alteration of the sperm count may not reflect the testis weight [25–27].

Interestingly, testis weight increased in the exposed group at 14 μ T MF for 16 weeks compared to that in sham control group, while it remained unaffected in mice exposed to the 200 μ T, 0.1 and 0.5 mT MF for 8 weeks [25, 26]. No significant association between a decrease in mature spermatogenic cells and alteration of testis weight was observed for 8 weeks of ELF-MF exposure [25]. In another report, sperm counts decreased after MF exposure for 4 weeks without significant histopathological changes in the testis of mice, though the testicular weight was significantly lower than that of the control [24].

In rats, ELF-MF can impair spermatogenesis recovery after heat-induced reversible testicular damage [52]. Ultrastructural changes in spermatogonia and spermatocyte occurred earlier than degeneration of Sertoli cells, suggesting that spermatogenic cells may be more sensitive to EMF exposure than Sertoli cells.

Stressful conditions to the testis, such as MF exposure, which induces early germ cell degeneration and a reduction of spermatogenesis, may however not be reflected in a reduced sperm counts in the ejaculation until months later.

For long-term exposure up to 46 weeks to ELF-MF of 0.1 or 0.5 mT, testis weight decreased in mice of the first and the second generations. The reduction rate of testis weight on the second generation decreased significantly by about 60%, compared with 10% in the first generation, whereas testis weight was unaffected in the third generation [19]. Testicular histological findings failed to show significant changes in the first-generation mice, while an increase of phagocytic cells and active spermatogenesis were observed in the gonads of the second-generation mice but not in those of the third-generation mice. These results suggest that long-term continuous exposure may induce adaptive mechanisms, which protect the DNA from harmful influences.

Table 2. Effects of ELF-MF on histomorphology and function of testis in rodents.

Animal	Age	MF (Hz)	Density	Exposure	Duration	Testis	Functional	Growth rate	Result	Ref.
Mice		50	6.4 mT	4 weeks	Continuous	Testis weight ↓ sperm ↓ motility ↓ morphology ↓ mature sperm cell ↓			S	24 (Hong et al, 2003)
BALB/c	7 weeks	60	0.1 or 0.5 mT	8 weeks	Continuous	Testis weight ↔			NS	25 (Lee et al, 2004)
BALB/c	18–26 gm	60	14 μT, 200 μT	16 weeks	Continuous	Testis weight ↑ at 14 μT, ↔ at 200 μT, germ cell apoptosis ↑ in exposed group			S	26 (Kim et al, 2009)
SD rat	12 weeks	50	100 μT, 500 μT	10 ms	2 h/day	Sperm count, morphology, apoptosis	Oxidative stress parameter		NS	32 (Akdag et al, 2013)
CD-1 mice	12 weeks	60	2 mT	72 h, 10 days	8 h/day	Meiotic chromosome aberration, sperm morphology			NS	33 (Heredia-Rojas et al, 2004)
SD rat	8 weeks	50	500 μT	4, 8 weeks	4 h/day	Testis weight, histology, oxidative status	T (↔)		NS	34 (Duan et al, 2014)
Wistar rat	Foetal	60	1 mT	13th gestation to 21st postnatal	30 min × 3/day	Histomorphologic parameter: seminiferous epithelium ↓, Leydig cell, connective tissue ↑	T (↔)	NS	S	47 (Tenorio et al, 2011)
CFLP mice	8–9 weeks	50	100 μT	14 days	23.5 h/day		HCG-stimulated T-response higher		S	89 (Forgács et al, 2004)
BALB/c	8 weeks		3 mT	8 weeks	4 h/day	1 spermatocyte, Leydig cell ↑	M1*/M2↓		S	103 (Ebrahimi-Kalan et al, 2011)
Wistar rat	180–200 gm	SMF	128 mT	30 days	1 h/day	Spermatogenesis ↔	T ↓, DNA oxidation (+)		S	104 (Amara et al, 2006)
NS, not significant; S, significant; *M, methionin.										

4.1. Intermittent exposure

Intermittent exposure of ELF-MF may lead to chromosomal damage in dividing cells [53]. A negative result was reported in a study regarding the genotoxicity of ELF-MF performed at continuous exposure [54, 55].

Several possible mechanisms may explain why intermittent ELF-MF can induce genotoxicity, including micronuclei formation [56], chromosomal aberrations in human amniotic cells [57], induction of DNA strand breakage in cultured human fibroblast [7] or dose-dependent DNA damage [58]. In contrast to continuous ELF-MF exposure, the application of intermittent MF results in a significant increase of DNA damage. Nonetheless, a major limitation is that the most results suggesting a genotoxic effect of intermittent MF were obtained from *in vitro* studies.

Reference/ Species	Intensity	Exposure time (weeks)																	
		1	2	4	6	8	12	16	18	20									
[16] Mice	50 Hz, 0.2/ 3.2/6.4 mT	Abnormal sperm ↑, 1C ↑, S phase ↑			Sperm ↓ Twt ↓														
[17] BALB/c	60 Hz 0.1, 0.5 mT						Twt ↔, Sperm ↓ Late apoptotic spermatogonia												
[18] BALB/c	60 Hz/ 14, 200 μT									Twt (14 μT ↑, 200 μT ↔), T ↔ Apoptosis ↑ (Spermatogonia)									
[19] BALB/c	60 Hz 20, 100 μT			Apoptosis ↑						Twt ↔ Apoptosis ↑									
[21] Hamster	0.1 mT One time (15 min) Intermittent (1 hr/D)	MEL ↓		16 ds MEL ↓, PRL ↑		Twt ↓ MEL, PRL ↔													
[22] SD Rat	50 Hz/ 25 μT					T ↓												LH ↑, T ↔ FSH ↔, Twt ↔, Sperm ↓	
[23] Rat	50 Hz/ 5 mT	FSH ↑				T ↔, LH ↑, PRL ↑													
[25] Rat	500 μT 4 h/day intermittent			T ↔ Sperm count ↔			T ↔ Sperm count ↔												
[50] Rat	60 Hz/1 mT after heat shock			15 d, Spermatogonia Spermatocyte apoptosis						60 d, Sertoli cell degeneration									
[61] Mice	50 Hz 1.7 mT	2 hrs exposure 4 hrs exposure				- No effect - Elongated spermatid ↓													
[62] Mice	50 Hz, 1 mT		T ↑		1C/4C ↑ (Meiosis) 4C, 4C/2C ↓		1C, 1C/2C ↑ (Germ cell transformation) 2C ↓ (Spermatogonia)												
[94] Rat	50 Hz/ 1, 5, 50 μT					T ↔													
[96] Rat	50 Hz, 2 hr/day Intermittent			10 days, Leydig cell proliferation T ↑, Spermatogenic cell ↓															
[97] Rat	50 Hz/0.8 mT 3h/day intermittent					T ↑ Abnormal sperm ↑													
[101] Mice	50 Hz/ 0.2, 6.4 mT			DNA migration ↑ Dose-dependent															

Twt: Testis weight, T: Testosterone, MEL: Melatonin, PRL: Prolactin, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone

Figure 1. Biological effects of ELF-EMF exposure in rodent male reproductive function.

Moreover, the results of intermittent exposure to ELF-MF are inconsistent. Cultured human diploid fibroblasts exposed intermittently to ELF-MF of 50 Hz at 1 mT presented a significant increase of DNA damage, in contrast to the recorded in a continuous ELF-MF exposure [7]. The highest level of induced DNA damage occurred at 5-min fields-on/10-min fields-off, among various intermittent exposure conditions. The results suggest that more than 10-min

extended off-time may give time for recovery. However, in rat studies, the intermittent exposure to 50-Hz ELF-MF of 500 μ T (the European reference level for occupational exposure) had no adverse effects on spermatogenesis applied 4 h per day for 4 or 8 weeks [34]. In addition, there were no significant differences between ELF-MF-exposed rats and sham controls regarding parameter for oxidative stress. Other studies showed that exposure to intermittent ELF-MF reports no significant effects on sperm morphology, meiotic chromosome aberration after 2 mT MF for 72 h or 10 days, nor on sperm parameters and germ cell apoptosis after 100 or 500 μ T, 2 h per day for 10 months [32, 33]. It suggests that relatively low intensity and short-term exposure to EMF would not be significant risk factors on spermatogenesis. Figure 1 lists the reported biologic effects on testis function in animals exposed to ELF-MF.

5. Germ cell apoptosis and ELF-MF exposure

Apoptosis, also called programmed cell death, is a key phenomenon in the control of sperm production. It is suggested that surplus cells and genetically abnormal cells are spontaneously eliminated by apoptosis as a defense mechanism during spermatogenesis [36]. The regulation of germ cell apoptosis during spermatogenesis is mediated by Sertoli cell-derived signals over each germ cell to which it is closely associated. Spontaneous apoptosis and pathological increase of germ cell death are induced by various external stimuli including exposure to heating, deprivation of gonadotropin and testosterone and chemotherapeutic agents [59–62]. The mechanisms of germ cell apoptosis triggered by exposure to ELF-MF are not well understood; however, it is considered to be different from those induced by aging [60], heat or hormonal deprivation [61, 62].

In mice, spontaneous apoptosis is most commonly observed in spermatocytes, including dividing spermatocytes, whereas the apoptotic rate in spermatogonia is significantly lower [63]. Histological characteristics of the seminiferous epithelium correlated with aging in rats indicate a decrease in the proliferation of spermatogonia and an increase in spermatogonia apoptosis [60]. Apoptosis associated to heat or testosterone treatment occurs mainly in round spermatids and pachytene spermatocytes [64, 65]. In mouse testis irradiated with single doses of γ rays, ionising radiation of up to 5 Gy, marked changes of testicular histology were induced by even 0.5 Gy. An apoptosis was characterized by a rapid onset of degeneration of spermatogonia and preleptotene spermatocyte [66]. However, the typical morphological characteristics of apoptosis, such as margination of chromatin or nuclear fragmentation, are rarely seen. Apoptosis related to androgen withdrawal predominantly affects spermatocytes and round spermatids [67].

Prominent histopathological alteration in testes exposed to ELF-MF showed an increased frequency of germ cell apoptosis and a decrease of mature spermatogenic cells, especially sperm [25–27]. In ELF-EMF-exposed mice, main TUNEL-positive cells are spermatogonia [25, 26] (Fig. 2).

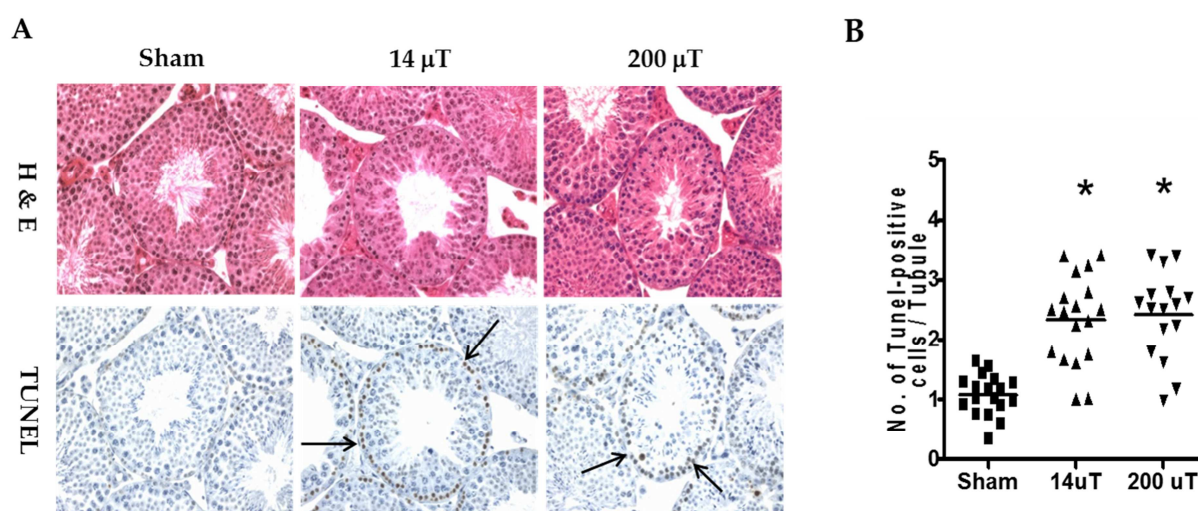


Figure 2. Effects of exposure to 60-Hz EMF on testicular germ cells apoptosis. A. Routine haematoxylin and eosin (upper row) and TUNEL (bottom row) staining of cross-sectioned seminiferous tubules. Most TUNEL-positive cells (arrow) were spermatogonia. (Magnification: $\times 400$). B. Frequency of apoptosis was increased in mice of exposed groups. The data are mean \pm S.E. * $P < 0.001$ vs. sham control. Adapted from Kim YW, Kim HS, Lee JS, Kim YJ, Lee SK, Seo JN, et al. Effects of 60 Hz 14 μ T magnetic field on the apoptosis of testicular germ cell in mice. *Bioelectromagnetics*. 2009;30:66–72.

The continuous exposure to a 60-Hz MF may affect biological processes including apoptotic cell death and spermatogenesis in the male reproductive system of mice in duration- and dose-dependent manner [27]. The continuous exposure to ELF-MF of 0.1 or 0.5 mT for 8 weeks induced testicular germ cell apoptosis in BALB/c mice [25]. A significant increase in the incidence on testicular germ cell death was referred, although non-significant body or testis weight was recorded. The continuous exposure to a 60-Hz MF at 100 μ T for 8 weeks or at 14 μ T for 16 weeks induced testicular germ cell apoptosis in mice [27] (Fig. 3). The minimum dose to induce apoptosis in testicular germ cell in mice was less than 20 μ T at continuous exposure to a 60-Hz MF for 8 weeks and the minimum duration was 6 weeks at continuous exposure of field strength 100 μ T.

5.1. Flow cytometric analysis

Flow cytometric analysis showed that in mice exposed to 60-Hz MF of 0.1 mT or 0.5 mT for 8 weeks, an increase in late apoptosis of testicular germ cells was originated [25]. Moreover, the testicular biopsy score showed a significant decrease in mature spermatogenic cells or spermatozoa in exposed mice without concurrent significant effect on the testis weight. It has been accepted that there was a high correlation between the testicular biopsy score and sperm count [68]. Flow cytometric studies in mice exposed to ELF-MF of 6.4 mT for 2 weeks revealed a significant decrease in mature spermatogenic cells (round spermatids, 1C) compared to the controls [24], whereas the differentiating spermatogonia cells (S phase) were significantly increased. After 4-week exposure, the testis weight of exposed mice was significantly lower compared with control, although no significant changes in the percentage of spermatogonia (2C) or primary spermatocyte (4C) were observed. These results suggest a possible cytotoxic

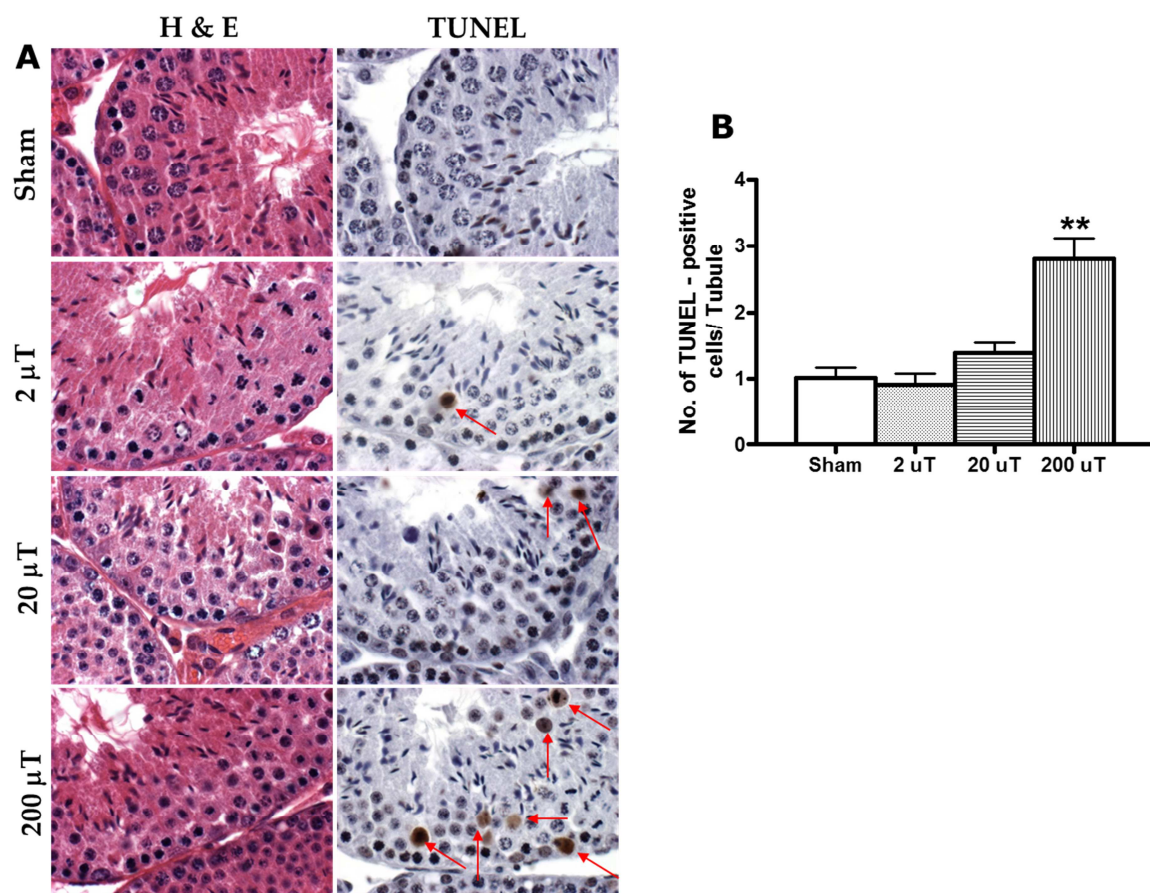


Figure 3. Effects of a 60-Hz MF on testicular germ cells apoptosis in mice exposed for 8 weeks. A. Routine haematoxylin and eosin (left) and TUNEL (right) staining of cross-sectioned seminiferous tubules. (Magnification: $\times 400$). B. Frequency of apoptosis dose-dependent increased in exposed groups. The data are means \pm S.E. ** $P < 0.001$ vs. sham control. Adapted from Kim HS, Park BJ, Jang HJ, Ipper NS, Kim SH, Kim YJ, et al. Continuous exposure to 60 Hz magnetic fields induces duration- and dose-dependent apoptosis of testicular germ cells. *Bioelectromagnetics*. 2014;35:100–107.

effect on differentiating spermatogonia. Moreover, a decrease in testis weight could be related to early loss of mature spermatogenic cells. A 28 days of exposition to 50-Hz EMF of 1.7 mT, in mice, had no effects when exposition was limited to 2 h, but when exposition was lengthened to 4 h, a significant decrease in elongated spermatids was observed [50]. Mice exposed to 50-Hz MF of 1.0 mT for 52 days presented a significantly higher total germ cell transformation and lower spermatogonia population compared to the corresponding control groups [69]. In summary, flow cytometric analysis shows that long-term exposure to EMF-MF has a possible effect on apoptosis of mature spermatogenic cells and a differentiation of spermatogonia.

6. Genotoxic effect of ELF-MF exposure

MF of very high intensity clearly induces adverse biological effects. However, time-varying ELF-MF is too weak to break DNA strands. Still, literature review on the genotoxic potential

of electric and magnetic fields demonstrated that ELF-MF might cause genotoxic effects [55]. The International Agency for Research on Cancer (IARC) concluded that ELF-MF might be carcinogenic to humans based on evidences associating residential exposure to MF with twice the risk for childhood leukaemia in children exposed to more than 0.4 μ T [10, 70].

In regards to the mechanism by which ELF-MF induce DNA damage, it was suggested that MF could act as a co-inducer of DNA damage rather than as a genotoxic agent *per se*. Genotoxic effects of EMF may occur indirectly by the generation of oxygen radicals or impairment of a radical scavenging mechanism [71]. *In vivo* studies showed a dose-dependent increase in DNA strand breaks following acute (2 h) 60-Hz MF exposure at exposure levels ranging from 0.1 to 0.5 mT in rat brain cells [72]. In human diploid fibroblasts *in vitro*, intermittent exposure (5 min on/10 min off) to ELF-MF results in a significant increase of DNA strand breakage in contrast to continuous exposure [7]. The results suggest that intermittent MF exposure may defer the adaptive mechanism indicating that ELF-MF-induced damage could be removed by the DNA repair mechanism. At extended off-times, no significant differences compared to the control were observed.

A recent review of on the topic including *in vivo* and *in vitro* studies related to the induction of DNA strand breakage by ELF-MF exposure highlighted conflicting evidences [73]. Proposed biological and biochemical responses of EMF effects are variable, including those on cell proliferation [6, 7], cell shape, modification of cell membrane structure [8], alteration of gene expression [9], induction of apoptosis [10] or production of reactive oxygen species (ROS) [70, 74, 75]. Reported changes in cell proliferation patterns [76, 77], alteration of Leydig cells and pineal gland function [78, 79] may also be related to genotoxic effects.

6.1. Free radicals and EMF exposure

Results on genotoxic effects of ELF-MF are contradictory. Several mechanisms have been proposed to explain DNA damage by indirect actions related to ELF-MF exposure. The possible biological mechanism of interaction involves the alteration of the cell membrane as the target for field interaction [80], disruption of the membrane protein, which may be affected by MF [81], or free radical-mediated damages on macromolecules [73, 82]. The changes of redox state induced by disturbed oxidative stress are related to cell cycle disturbance [83].

In biological systems, free radicals are produced by normal metabolism and electron transfer reaction in the cell membranes, mainly in the mitochondria membrane [80]. The balance between ROS production and antioxidants capacity can be disturbed by external stressors, such as exposure to MF or chemical agents. Modulation of antioxidants by ELF-MF can impair the intracellular defense mechanism inducing the development of DNA damage, which may be related to cancer development. The investigation for a correlation between exposure to ELF-MF and an increased incidence of tumours is however contradictory. The modulation of cellular redox balance is affected by the enhancement of an oxidative intermediate, or the inhibition or reduction of antioxidants. Those may be influenced by environmental factors such as ELF-MF [84]. EMF-MF might compromise the intracellular defense activity promoting the development of DNA damage. Exposure of the cell to 50-Hz MF and simultaneous

treatment with an oxidant may affect the DNA damage [85]. As DNA damage is not repaired, a nuclear enzyme triggers apoptosis. Moderate oxidative stress induced apoptosis, whereas a higher dose of ROS initiated cell necrosis [86]. It seems that EMF enhances the physiologic functions such as activation of certain cell types. ELF-MF affects gene transcription, cell growth and apoptosis, as well as the membrane-mediated signal transduction process [9]. Therefore, although ELF-MF may be weak, it may affect various biological functions of living organs.

7. Cell proliferation and EMF exposure

There are several *in vitro* studies related to cell differentiation under EMF exposure conditions [76, 77, 87]. Cell proliferation and DNA synthesis are related to the initial induction of mutation that eludes the physiologic defense system and DNA repair. In contrast to such biological effects of ELF-MF exposure, there are beneficial effects on tissue healing in bone fracture regarding ELF-MF exposure [88]. It has been shown that ELF-MF influence proliferation and DNA damage in both normal and tumour cells *in vitro* through the action of free radical species [77].

It was reported a dose-dependent increase in the proliferation rate in certain cell types, namely the HL-60 leukaemia cells and rat fibroblast, exposed to ELF-EMF, followed by the simultaneous increase in DNA strand breakage and in 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation, one of the prominent forms at lesion of radical-induced DNA damage. The effects of ELF-MF on cell proliferation and DNA damage were prevented by antioxidant treatment [77].

Another *in vitro* study refers to mutations in the hypoxanthine-guanine phosphoribosyl transferase gene as induced in human osteosarcoma cell line and in Chinese hamster ovary cells (CHO) after exposure to ELF-MF [76]. An increase of human chorionic gonadotropin (HCG)-stimulated testosterone production was observed after exposure of 50-Hz MF in a mouse primary Leydig cell culture [89]. Other study revealed that EMF exposure originated the stimulation of Ca^{2+} influx in rat pituitary cells, as part of the regulatory process in the Leydig cell steroidogenesis [90]. Modulation of calcium signalling by EMF was pointed as a possible candidate for activation of biochemical reactions [91]. On the other hand, it could also be possible that an alteration in cAMP contents and in intracellular communication might be associated with its effects on steroidogenesis [92].

Exposition of cryptorchid rats to intermittent EMF stimulation for 10 days induced Leydig cell proliferation, along with an increase in plasma testosterone and in testis weight [93]. ELF-MF exposure increased the HCG-stimulated capacity for testosterone production in mice Leydig cells *ex vivo* [89]. Since they may not be hormonally mediated, it was hypothesised that the possible biological effects on Leydig cells would involve direct cytotoxic effects [79]. In another study, a significant increase in the size and weight of testes was related to an increase in the amount of interstitial tissue. Elevated testosterone levels after a 10-week exposure to 50 Hz of 100 μT MF was also observed [94].

The percentage of cells in S phase significantly increased in mice exposed to 6.4 mT, with a subsequent decrease in sperm count and testis weight [95]. Mice exposed to 50 Hz for 52 days also evidenced increased total germ cell transformation, the spermatogonia cell population being significantly lower than in the corresponding control [69]. These results suggest that long-term exposure to ELF-MF has possible effects on the proliferation and differentiation of spermatogonia.

8. Hypothalamic–pituitary–gonadal axis and EMF exposure

Spermatogenesis is controlled by the hypothalamic–pituitary–gonadal (HPG) axis. In the model of hormonal deprivation, hypophysectomy-associated cell loss in the testis results from germ cell apoptosis [61]. Several studies report a suppression of melatonin production in the pineal gland of rats as a consequence of EMF exposure [29, 80, 96, 97]. Acute MF exposure can result in altered pineal gland and HPG function. One-time or intermittent exposure to 60-Hz MF at 0.1 mT is associated with a reduction in melatonin concentration. Daily intermittent exposures for 16 days increases prolactin levels and suppresses normal nocturnal rise in pineal melatonin production. However, at 42 days, there is no significant effect in melatonin or prolactin levels [29]. It suggests that pituitary–gonadal axis may adapt to chronic exposure to EMF. A reduced circulating concentration of melatonin may result in an increased prolactin release but the pituitary stimulated estrogen and testosterone levels by the gonads [98]. It is proposed that melatonin might be considered essential to both spermatogenesis and folliculogenesis [99].

Testosterone is crucial for the spermatogonia differentiation into round or elongated spermatids. Deprivation of gonadotropin or testosterone induces germ cell apoptosis [61]. In rats or mice, despite a decrease in sperm count or increase in frequency of germ cell apoptosis, exposure to ELF-MF did not affect serum testosterone level [26, 30, 34, 100].

In rats, follicle-stimulating hormone (FSH) increased within 1 week and luteinizing hormone (LH) increased in 4 weeks after exposure to 50 Hz of 5 mT without significant changes in the peripheral testosterone levels [30]. Since FSH levels affects spermatogenesis, an elevated FSH level suggests the disturbance of the spermatogenic process. In rats, the seminiferous tubules with the maximal response to FSH are also those presenting higher spontaneous apoptosis in spermatogonia [101]. It was also consistently observed that mature spermatogenic cells, such as spermatid and sperm, decrease in a relatively early phase of EMF exposure [25, 26]. It has been hypothesised that the early histologic findings in testis after ELF-MF exposure—a decrease in mature spermatogenic cells, such as round and elongated spermatid—may stimulate FSH secretion in the hypophysis by a positive feedback. This would be followed by a decrease in testosterone synthesis due to possibly damage in Leydig cells. Testosterone levels are supposed to be partially recovered by up-regulation of pituitary gonadotropin. On the other hand, 13 days of short-period exposure to MF in mice lead to a rise in testosterone levels [69]. *In vivo* experiments showed that 2 h per day exposure for 10 days of EMF stimulation results in Leydig cell proliferation, an increase in testosterone level and testis weight but a decrease in germ cell population [93]. In rats, ELF-MF exposure for 5 weeks, 3 h per day in 50

Hz of 0.8 T MF originated an increase in testosterone levels [102]. It seems that the testosterone transiently increases in the early phase of MF exposure due to Leydig cell proliferation followed by a decline of testosterone production related to damage in Leydig cells induced by MF exposure.

In mice, exposure to 50 Hz of 100 μ T for 48 h originates a markedly increase in the steroidogenic capacity of Leydig cells without alterations in the serum testosterone level or in the testicular histology [79]. The results suggest that the effect of MF exposure on mouse Leydig cells and an alteration in testosterone level might not be mediated by gonadotropin. In Sprague-Dawley rats exposed to 50 Hz at field strength 25 μ T, testosterone levels were significantly decreased only after 6 and 12 weeks of exposition, which was followed by a significant increase in the serum levels of LH after 18 weeks of exposure, the FSH levels remaining unaffected [31]. It was proposed that an MF-induced decline in testosterone level would stimulate the HPG axis with positive feedback.

Testosterone level in mice exposed to EMF for 16 weeks was not modified despite the marked increased germ cell apoptosis [26]. Differentiating spermatogenic cell apoptosis may occur in the early phase of ELF-MF exposure without alteration in the peripheral testosterone level [25, 27, 52], supporting the idea that the biological effect of MF exposure on germ cell apoptosis may not be hormonally mediated.

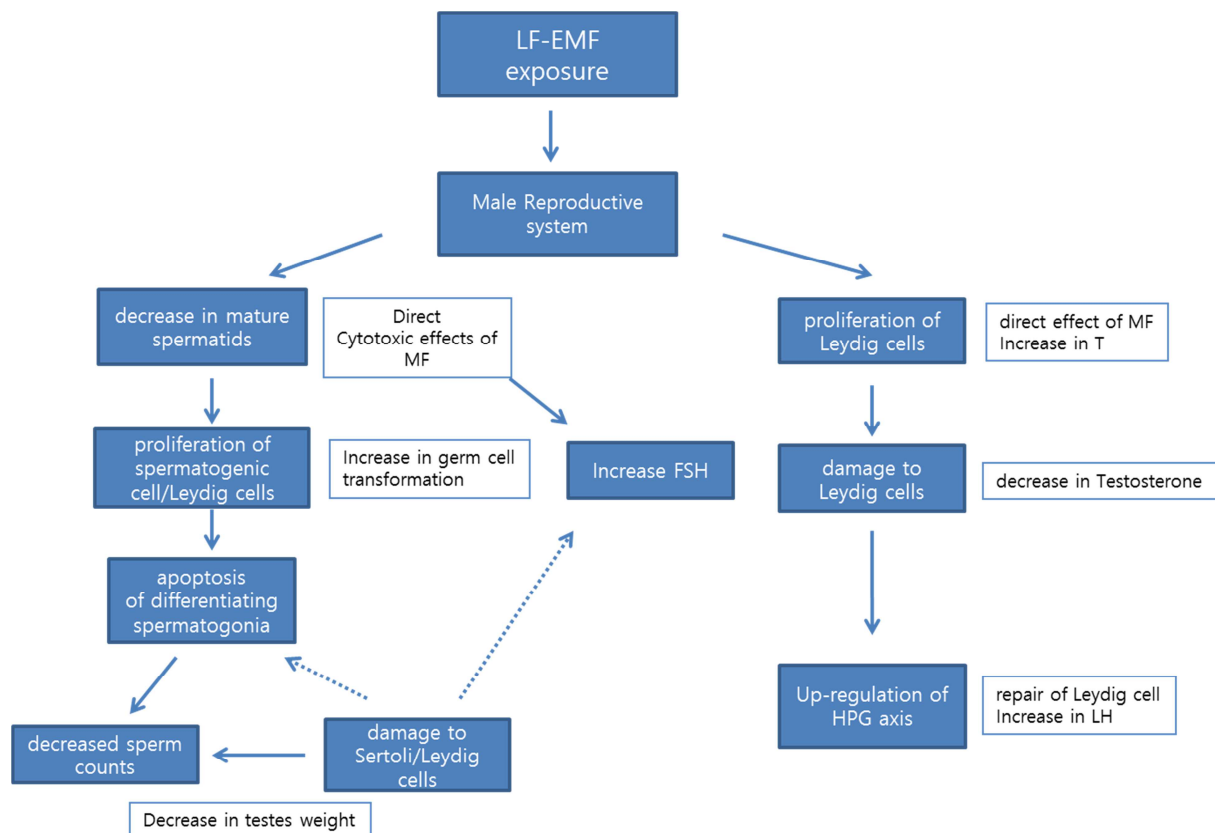


Figure 4. Diagram of the proposed mechanism for apoptosis in testis exposed to ELF-EMF.

Summarizing, cellular proliferation of Leydig cells may be induced at a relatively early phase after ELF-MF exposure. By consequence, testosterone production transiently increased, that is afterwards followed by a decrease in testosterone production due to disturbance of Leydig cell function, which in turn may stimulate LH production [19, 35]. However, the damaged Leydig cells induced by MF exposure may be repaired, in spite of germ cell death. The susceptibility to biological action of ELF-MF may differ according to the cell type [59] and Leydig cells may be more resistant to EMF exposure than germ cells (Fig. 4).

9. Summary and conclusion

A high-intensity MF with thermal effects is clearly teratogenic in laboratory and animal studies. A 50/60-Hz ELM-MF generated by power lines or an electric appliance is too weak, however, to induce DNA strand breakage. Nevertheless, studies regarding genotoxicity demonstrate that ELF-MF with a non-thermal exposure level is related to DNA damage in biologic systems. It is suggested that ELF-MF may act as a co-inducer to potentiate a suboptimal mutagenic signal.

Gonadal tissue involving germ cell differentiation and development is sensitive to external stressors, such as radiation, heat and exposure to chemical or physical agents. Putative harmful effects of MF on reproductive function have emerged as a major concern.

No consistent evidences exist on the adverse effects of ELF-MF on reproduction. To date, scientific literature reveals no significant risk on implantation or foetal development, but to minor skeletal alterations, an increased frequency of germ cell apoptosis in ELF-MF-exposed mice or rats has been consistently described. It was also suggested that a field of up to 20 mT does not increase a gross external or skeletal anomaly.

Accumulated evidence showed that ELF-MF exposure is cytogenetic to gonadal cells in rodents in a dose- and duration-dependent manner. Spermatogenic cells may be more sensitive to MF exposure than Leydig or Sertoli cells in testes. The pathway of testicular germ cell apoptosis following ELF-MF exposure is not well established. Based on relevant studies, germ cell apoptosis may be directly triggered by ELF-MF and not be hormonally mediated. However, chronic ELF-MF exposure disturbed the HPG axis. Testicular histology revealed alterations on Leydig cells producing testosterone and Sertoli cells supporting spermatogenesis in long-term MF-exposed mice.

Continuous exposure to ELF-MF in mice induces apoptosis of spermatogenic cells especially in mature spermatid, in a dose- and duration-dependent manner. For inducing apoptosis of testicular germ cells in mice, the minimum dose is represented by a field strength of 20 μ T at continuous exposure to 60-Hz MF for 8 weeks or by a minimum duration of 16 weeks at continuous exposure to 100 μ T, whereas intermittent exposure to ELF-MF, as low as 70 μ T, induces genotoxic effects *in vitro* [7]. Furthermore, intermittent exposure to MF might be more harmful to living cells than continuous exposure.

The magnitude of the biologic effects depends on the density of the magnetic fields, the duration of exposure and the time of recovery. It may be a dynamic compensatory mechanism

of spermatogenesis during germ cell apoptosis responding to exposure to ELF-EMF according to the intensity of EMF, the exposure pattern and duration. Long-term effects of chronic exposure have been excluded from the ICNIRP guidelines because of insufficient consistent scientific evidence to set a threshold for such possible adverse effects. According to the latest ICNIRP guideline for low-frequency MF (1 Hz to 100 kHz), the safety levels at short-term exposure are 1 mT for occupational exposure and 200 μ T for the general population [11]. Those safety levels are two-fold higher in the field density than those in the previous guideline. Until now, safety levels for long-term exposure are not determined.

Adverse effects of ELM-MF are mainly from animal experiments. The experimental MF exposure condition may generally differ from those found in the environment in real life. The stochastic probability of the occurrence of the biologic effects to a certain ELM-MF exposure level should be determined for risk assessment. For a better understanding of the mechanism regulating biological effects of ELM-MF, molecular signalling pathway needs to be elucidated.

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